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Expansion of response surface models for the growth of *Escherichia coli* O157:H7 to include sodium nitrite as a variable

Abstract

The previously published (Buchanan et al., 1993a) response surface models for estimating the aerobic and anaerobic growth of *Escherichia coli* O157:H7 as a function of temperature, initial pH, and sodium chloride content have been expanded to include sodium nitrite as a further variable. A fractional factorial design was employed to quantitate the effect of $NaNO_2$ in conjunction with the four other variables by culturing a three-strain mixture in brain heart infusion broth. The activity of $NaNO_2$ was strongly pH-dependent, with inhibition being significant at pH values ≤ 5.5 and enhanced by lowering the incubation temperature. The effects of the variables on *Escherichia coli* O157:H7 growth kinetics were modeled by response surface analysis using quadratic and cubic polynomial models of the natural logarithm transformation of both the Gompertz *B* and *M* parameters (Gompertz parameters) and the lag phase duration (LPD) and generation time (GT) values (kinetics parameters) calculated for individual growth curves. All models provided reasonable estimates for most variable combinations; however, comparisons of predicted versus observed values indicated that overall the most useful models were the cubic models based on LPD and GT values. Although additional validation of the models is required, comparisons of predicted times to a 1000-fold increase in population density against those calculated from previously published growth studies indicate that the models are an effective means for acquiring 'first estimates' of the growth characteristics of *E. coli* O157:H7.

The ability of certain *Escherichia coli* serovars, particularly O157:H7, to cause haemorrhagic colitis and hemolytic uremic syndrome is well established, with ground beef being one of the most commonly implicated foods (Padhye and Doyle, 1992). As part of a program to better characterize the behavior of *Escherichia coli* O157:H7 in foods, we previously assessed the effects of three variables, storage temperature, initial pH, and sodium chloride content (water activity), on the microorganism's aerobic and anaerobic growth kinetics (Buchanan and Klawitter, 1992). These quantitative data were then used to develop a set of response surface models for estimating the microorganism's growth as a function of the variables (Buchanan et al., 1993a). Since most *E. coli* O157:H7 outbreaks have involved meat products, it was decided to further investigate the pathogen's growth characteristics by determining its response to sodium nitrite. Under specific conditions, this curing agent possesses antimicrobial activity against a variety of foodborne pathogens. The data were then used to expand our response surface models to include sodium nitrite as a variable. A secondary objective of the study was to evaluate the relative effectiveness of models developed using the parameters of the modified Gompertz equation compared to models based on derived growth kinetics terms.

2. Materials and methods

Microorganisms. Three *Escherichia coli* O157:H7 strains (933, 45753-35, and A9218-C1) were used throughout the study. Working stock cultures were maintained in Brain Heart Infusion broth (BHI) (Difco) stored at 4°C and transferred monthly.

Experimental design. A fractional factorial design was employed to determine the effects of sodium nitrite (0–200 μg/ml) in conjunction with four other variables: temperature (5–42°C), initial pH (4.5–8.5), sodium chloride (5–50 g/l), and oxygen availability (aerobic versus anaerobic). The number of replicate cultures examined for each variable combination is indicated in Tables 1 and 2. These data were combined with the data set of Buchanan and Klawitter (1992) and Buchanan et al. (1993a).

Culture techniques. Except for the addition of sodium nitrite, the culture techniques were identical to those described previously (Buchanan and Klawitter, 1992; Buchanan et al., 1993a). Stock solutions of sodium nitrite were prepared prior to the initiation of each experiment by dissolving 0.5 g of $NaNO_2$ in 50 ml of distilled water. The solutions were sterilized by filtration (0.22 μm), and 0.25, 0.5, 0.75 and 1.00-ml aliquots transferred aseptically to the previously autoclaved and cooled media to achieve final concentrations of 50, 100, 150 and 200 μg/ml.

Curve fitting. Growth curves were generated by fitting the modified Gompertz function to the data as described previously (Buchanan and Klawitter, 1992; Buchanan et al., 1993). The Gompertz parameters were then used to calculate lag phase durations (LPD), exponential growth rates (EGR), generation times (GT), maximum population densities (MPD), and estimated times to 1000-fold increase in population density (t_{1000}).

Model generation. The effects of the four independent variables (temperature, pH, NaCl, and $NaNO_2$) on *E. coli* O157:H7 growth kinetics were modeled by response surface analysis using quadratic and cubic polynomial models of natural logarithmic transformations (Ln) of the Gompertz M and B parameters. Separate models were generated for the aerobic and anaerobic data sets. The Gompertz A and C parameters were not modeled based on previous observations and assumptions (Buchanan et al., 1993a). For comparisons of predicted versus observed growth kinetics, an A parameter was assumed to be 3.00 for both aerobic and anaerobic data sets. The C-term was assumed to be 6.40 and 5.81 for the aerobic and anaerobic cultures, respectively. The C-values were based on the grand means of the MPD values for the data sets and the relationship, MPD $= A + C$.

The effects of the independent variables were also modeled by submitting the LPD and GT values calculated for each growth curve to response surface analysis. Initial analyses compared models developed using untransformed data, as well as Ln and square root transformations. Models based on the Ln-transformation of LPD and GT were found to be consistently superior, and subsequent analyses were limited to this transformation. All model generation and additional statistical analyses were performed using SAS/STAT (SAS, 1989).

3. Results and discussion

A total of 66 aerobic (Table 1) and 71 anaerobic (Table 2) growth curves were generated, representing 50 and 53 unique variable combinations, respectively. These data were added to that of Buchanan and Klawitter (1992) and Buchanan et al. (1993a) so that 260 aerobic and 216 anaerobic growth curves representing 134 and 124 unique variable combinations, respectively, were available for model development. The complete data set is available upon request.

Qualitatively, the effects of sodium nitrite on the growth of *E. coli* O157:H7 were similar to those observed with other non-spore forming bacteria (Buchanan and Phillips, 1990; Zaika et al., 1991, 1992; Palumbo et al., 1991, 1992; Buchanan et al., 1993b). Its activity was strongly pH-dependent. For example, Fig. 1 depicts the effect of initial pH for 28°C-0.5% NaCl cultures grown aerobically and

Table 1
Variable combinations for which aerobic growth curves of a three-strain mixture of *Escherichia coli* O157:H7 were generated

TEM	pH	NaCl	NO_2	*n*	*B*	*M*	EGR	GT	LPD	MPD
12	5.5	20	200	1	0.0000	–	0.000	–	–	–
12	5.5	35	50	1	0.0000	–	0.000	–	–	–
12	6.5	5	0	1	0.0280	54.4	0.071	4.2	18.7	10.0
12	6.5	5	50	1	0.0282	61.3	0.068	4.4	25.8	9.7
12	6.5	5	100	1	0.0317	59.1	0.077	3.9	27.5	9.7
12	6.5	5	150	1	0.0292	56.5	0.069	4.3	22.3	9.4
12	6.5	5	200	1	0.0161	116.7	0.040	7.6	54.6	9.7
12	6.5	20	200	1	0.0141	111.4	0.023	13.3	40.5	7.4
12	7.5	5	200	1	0.0195	102.8	0.051	5.9	51.5	10.0
12	7.5	20	200	1	0.0164	109.5	0.035	8.6	44.5	9.1
19	5.5	5	50	2	0.0969	24.9	0.216	1.4	13.8	9.3
19	5.5	5	100	1	0.0400	69.9	0.103	2.9	44.9	10.1
19	5.5	5	150	1	0.0000	–	0.000	–	–	–
19	5.5	5	200	1	0.0000	–	0.000	–	–	–
19	6.5	5	50	1	0.0795	17.7	0.199	1.5	5.1	9.9
19	6.5	5	100	1	0.0989	24.8	0.245	1.2	14.7	9.9
19	6.5	5	150	1	0.0783	18.4	0.192	1.6	5.6	9.5
19	6.5	5	200	1	0.0778	18.7	0.187	1.6	5.8	9.5
19	7.5	5	100	1	0.0908	20.1	0.228	1.3	9.1	9.9
19	7.5	50	200	1	0.0182	102.0	0.039	7.7	47.1	8.8
28	4.5	5	200	1	0.0000	–	0.0000	–	–	–
28	5.5	5	200	1	0.1125	48.4	0.257	1.2	39.5	9.4
28	5.5	20	100	1	0.3124	25.2	0.640	0.5	22.0	8.7
28	5.5	35	50	1	0.2802	25.1	0.656	0.5	21.6	9.3
28	6.5	5	50	1	0.2811	6.2	0.667	0.5	2.7	9.6
28	6.5	5	100	1	0.2107	6.2	0.494	0.6	1.5	9.5
28	6.5	5	150	1	0.2754	6.2	0.641	0.5	2.7	9.5
28	6.5	5	200	3	0.2593	6.1	0.565	0.5	2.2	9.1
28	6.5	20	150	2	0.1662	9.3	0.421	0.7	3.1	9.6
28	6.5	35	100	2	0.1898	12.1	0.459	0.7	6.8	9.5
28	6.5	35	150	1	0.1710	11.7	0.432	0.7	5.8	10.0
28	6.5	50	50	3	0.0915	18.9	0.228	1.3	8.0	9.6
28	6.5	50	100	3	0.0829	21.1	0.201	1.5	9.1	9.7
28	6.5	50	150	3	0.1126	18.5	0.261	1.2	9.7	9.4
28	6.5	50	200	3	0.1924	20.8	0.426	0.7	15.6	9.2
28	7.5	50	200	2	0.1121	26.1	0.269	1.1	17.1	9.5
28	8.5	5	200	2	0.1545	8.0	0.361	0.9	1.2	9.5
37	5.5	5	200	1	0.1377	11.6	0.340	0.9	4.4	9.0
37	5.5	50	150	1	0.0888	73.1	0.164	1.8	61.8	8.1
37	6.5	5	50	1	0.3842	4.3	0.873	0.3	1.7	9.4
37	6.5	5	100	1	0.3859	4.5	0.879	0.3	1.9	9.4
37	6.5	5	150	1	0.3510	4.4	0.804	0.4	1.6	9.4
37	6.5	5	200	1	0.3190	4.6	0.737	0.4	1.4	9.3
37	6.5	50	50	1	0.1728	23.0	0.375	0.8	17.3	9.0
37	6.5	50	100	1	0.2380	22.4	0.512	0.6	18.2	8.9
37	7.5	5	200	1	0.4255	3.7	1.053	0.3	1.4	9.8
37	7.5.	50	50	1	0.1598	14.2	0.361	0.8	7.0	9.0
37	7.5	50	200	2	0.1709	18.7	0.488	0.8	12.8	9.4
42	6.5	5	100	1	0.5512	3.8	1.278	0.2	2.0	9.4
42	6.5	5	200	1	0.4287	4.0	1.109	0.3	1.6	10.0

Table 1 (continued)

TEM, incubatation temperature (°C); NaCl, sodium chloride (g/l); $NaNO_2$, sodium nitrite (μg/ml); *n*, number of replicate cultures; *B*, Gompertz *B* parameter; *M*, Gompertz *M* parameter; EGR, exponential growth rate ([Log(cfu/ml)]/h); GT, generation time (h);LPD, lag phase duration (h); MPD, maximum population density [Log(cfu)/ml];

anaerobically in the presence of 0 and 200 μg/ml $NaNO_2$. Little inhibitory activity was observed at pH ≥ 6.5. However, at pH 5.5 there was significant bacteriostasis involving both an extension of the lag period and a decrease in the exponential growth rate, with the anaerobic cultures being inhibited to a greater degree. At pH 4.5, 200 μg/ml $NaNO_2$ was bactericidal, with both the aerobic and anaerobic cultures being inactivated within 54 h. While $NaNO_2$ elicited a bacteriostatic effect as evidenced by an extension of the lag phase and a depression of the growth rate, it did not greatly influence the maximum population density. If a culture initiated growth, it typically achieved a MPD of between 10^9 and 10^{10} cfu/ml (Tables 1 and 2).

The inhibitory activity of $NaNO_2$ was increased when the incubation temperature was lowered, as demonstrated in Fig. 2 which compares the t_{1000} times for pH 5.5-0.5% NaCl cultures containing 0 and 200 μg/ml $NaNO_2$. Sodium chloride affected the growth of *E. coli* O157:H7, particularly at low temperatures and pH values. However, NaCl appeared to have relatively little effect on enhancing the activity of sodium nitrite at pH values ≥ 6.5, particularly at optimal temperatures.

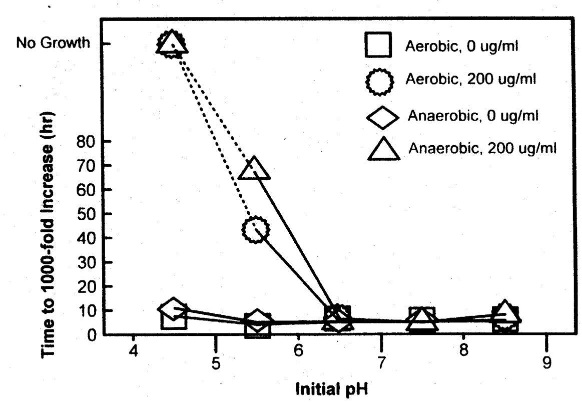

Fig. 1. Effect of initial pH on the time to achieve a 1000-fold increase in population density for 28°C-0.5% NaCl cultures of *Escherichia coli* O157:H7 grown aerobically and anaerobically in the presence of 0 and 200 μg/ml $NaNO_2$.

Table 2
Variable combinations for which anaerobic growth curves of a three-strain mixture of *Escherichia coli* O157:H7 were generated

TEM	pH	NaCl	NO_2	*n*	*B*	*M*	EGR	GT	LPD	MPD
12	5.5	20	200	1	0.0000	–	0.000	–	–	–
12	5.5	35	50	1	0.0000	–	0.000	–	–	–
12	6.5	5	0	1	0.0279	51.6	0.066	4.6	15.7	9.5
12	6.5	5	50	1	0.0250	56.0	0.060	5.0	16.0	9.7
12	6.5	5	100	1	0.0285	53.8	0.067	4.5	18.7	9.6
12	6.5	5	150	1	0.0330	54.6	0.070	4.3	24.3	8.8
12	6.5	5	200	1	0.0177	118.1	0.040	7.6	61.6	9.1
12	6.5	20	200	1	0.0000	–	0.000	–	–	–
12	7.5	5	200	1	0.0219	106.0	0.054	5.6	60.4	9.5
12	7.5	20	200	1	0.0164	113.2	0.035	8.7	52.3	8.7
19	5.5	5	50	1	0.0731	65.9	0.193	1.6	52.2	10.2
19	5.5	5	100	1	0.0383	59.6	0.098	3.1	33.5	9.8
19	5.5	5	150	1	0.0000	–	0.000	–	–	–
19	5.5	5	200	1	0.0000	–	0.000	–	–	–
19	6.5	5	50	1	0.1009	14.9	0.225	1.3	5.0	9.1
19	6.5	5	100	1	0.0926	15.7	0.214	1.4	4.9	9.2
19	6.5	5	150	2	0.0896	16.5	0.205	1.5	5.3	9.2
19	6.5	5	200	2	0.0774	20.1	0.180	1.7	7.0	9.4
19	7.5	5	100	1	0.0861	17.5	0.201	1.5	5.9	9.3
19	7.5.	50	200	1	0.0161	107.8	0.031	9.6	45.6	8.2
28	4.5	5	200	1	0.0000	–	0.000	–	–	–
28	5.5	5	200	1	0.0859	71.8	0.189	1.6	60.7	8.9
28	5.5	20	100	1	0.0935	48.2	0.213	1.4	37.5	9.2
28	5.5	35	50	1	0.0489	96.1	0.096	3.1	75.7	8.3
28	6.5	5	50	1	0.3022	5.9	0.661	0.5	2.6	9.1
28	6.5	5	100	1	0.1990	6.2	0.451	0.7	1.7	9.3
28	6.5	5	150	1	0.3077	7.6	0.692	0.4	4.4	9.3
28	6.5	5	200	3	0.1683	10.4	0.377	0.8	4.5	9.3
28	6.5	20	150	2	0.2038	9.9	0.466	0.7	5.0	9.2
28	6.5	35	100	2	0.1870	11.8	0.455	0.7	6.4	9.2
28	6.5	35	150	1	0.1677	10.9	0.395	0.8	4.9	9.5
28	6.5	50	50	3	0.1112	17.3	0.233	1.3	8.4	8.6
28	6.5	50	100	3	0.1240	18.3	0.247	1.2	10.2	8.5
28	6.5	50	150	3	0.1279	14.8	0.268	1.1	6.9	8.8
28	6.5	50	200	3	0.2055	21.5	0.401	0.8	16.6	8.3
28	7.5	5	200	1	0.2503	6.3	0.566	0.5	2.3	9.1
28	7.5	50	200	2	0.0951	21.6	0.212	1.4	11.1	9.0
28	8.5	5	200	1	0.1692	10.7	0.385	0.8	4.8	9.2
37	5.5	5	200	1	0.2134	38.1	0.444	0.7	33.4	8.6
37	5.5	50	150	1	0.0000	–	0.000	–	–	–
37	6.5	5	50	1	0.3891	4.1	0.814	0.4	1.5	8.8
37	6.5	5	100	1	0.3812	4.2	0.785	0.4	1.6	8.8
37	6.5	5	150	1	0.3958	4.7	0.834	0.4	2.2	8.8
37	6.5	5	200	1	0.3519	4.5	0.739	0.4	1.7	8.8
37	6.5	50	50	1	0.1754	12.1	0.378	0.8	6.4	8.8
37	6.5	50	100	2	0.1953	11.5	0.409	0.7	6.3	8.6
37	6.5	50	200	1	0.1448	13.2	0.315	1.0	6.3	8.9
37	7.5	5	200	1	0.4628	3.5	1.103	0.3	1.3	9.6
37	7.5	50	50	1	0.1386	11.6	0.326	0.9	4.4	9.1

Table 2 (continued)

TEM	pH	NaCl	NO_2	*n*	*B*	*M*	EGR	GT	LPD	MPD
37	7.5	50	100	1	0.1571	11.2	0.352	0.9	4.8	8.9
37	7.5	50	200	3	0.2114	14.9	0.447	0.7	9.6	8.8
42	6.5	5	100	1	0.4714	3.9	1.127	0.3	1.7	9.6
42	6.5	5	200	1	0.4096	4.8	1.086	0.3	2.4	10.3

See Table 1 for abbreviations.

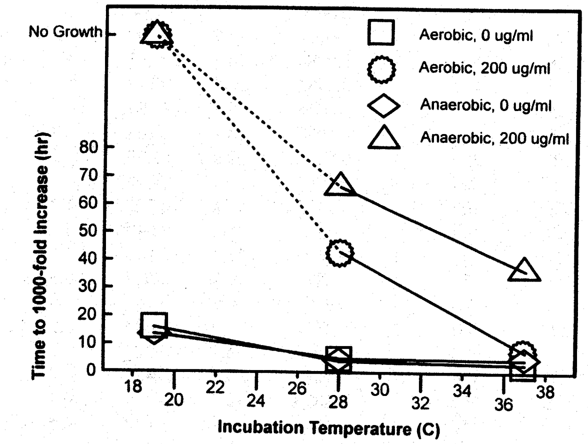

Fig. 2. Effect of incubation temperature on the time to achieve a 1000-fold increase in population density for pH 5.5-0.5% NaCl cultures of *Escherichia coli* O157:H7 grown aerobically and anaerobically in the presence of 0 and 200 $\mu g/ml$ $NaNO_2$.

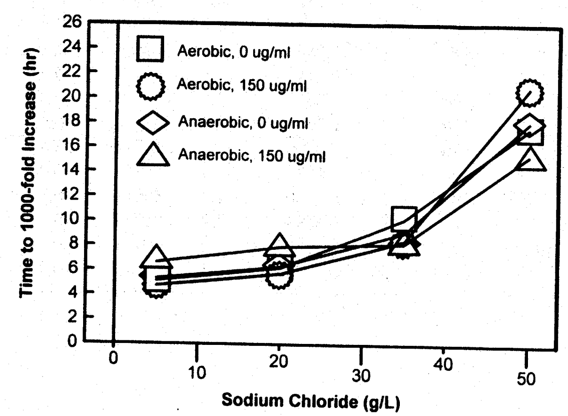

Fig. 3. Effect of sodium chloride concentration on the time to achieve a 1000-fold increase in population density for 28°C-pH 6.5 cultures of *Escherichia coli* O157:H7 grown aerobically and anaerobically in the presence of 0 and 150 $\mu g/ml$ $NaNO_2$.

Gompertz parameters

$$
\begin{aligned}
\mathrm{Ln}(B) = {} & -26.7466 + 0.7324T + 7.4535P - 0.297S - 0.0267N - 0.1108TP + 0.00454TS + 0.000293TN \\
& + 0.05PS + 0.0092PN + 0.000374SN - 0.00886T^2 - 0.8375P^2 + 0.00083S^2 - 0.000189N^2 \\
& - 0.000026TPS + 0.000017TPN + 0.00000268TSN - 0.0000513PSN + 0.000786T^2P \\
& - 0.0000414T^2S + 0.00000335T^2N - 0.00151P^2S + 0.00544TP^2 - 0.000926P^2N \\
& - 0.000471PS^2 - 0.0000387TS^2 - 0.00000494S^2N - 0.00000069TN^2 + 0.0000244PN^2 \\
& + 0.00000085SN^2 + 0.0000126T^3 + 0.0294P^3 + 0.0000414S^3 + 0.00000011N^3
\end{aligned}
$$

$r^2 = 0.894$

$$
\begin{aligned}
\mathrm{Ln}(M) = {} & 17.9641 - 0.386T - 4.2397P + 0.314S + 0.1212N + 0.069TP - 0.00111TS + 0.000235TN \\
& - 0.067PS - 0.0308PN - 0.000561SN - 0.000488T^2 + 0.4291P^2 - 0.00216S^2 \\
& - 0.0000695N^2 - 0.000435TPS - 0.0000277TPN + 0.00000369TSN + 0.0000748PSN \\
& - 0.000302T^2P + 0.0000199T^2S + 0.00000066T^2N + 0.00424P^2S - 0.00421TP^2 \\
& + 0.00181P^2N + 0.0004PS^2 + 0.0000638TS^2 - 0.00000067S^2N - 0.00000104TN^2 \\
& + 0.0000174PN^2 - 0.00000018SN^2 + 0.000073T^3 - 0.011P^3 - 0.0000208S^3 \\
& - 0.00000003N^3
\end{aligned}
$$

$r^2 = 0.947$

Kinetics parameters

$$
\begin{aligned}
\mathrm{Ln(LPD)} = {} & 18.2461 - 0.1626T - 5.0804P + 0.131S + 0.1603N + 0.053TP - 0.00134TS + 0.000919TN \\
& - 0.0187PS - 0.0368PN - 0.000962SN - 0.00925T^2 + 0.5434P^2 - 0.00374S^2 \\
& - 0.000296N^2 - 0.000609TPS - 0.0000995TPN + 0.00000777TSN + 0.000125PSN \\
& - 0.0000733T^2P - 0.00000972T^2S + 0.00000116T^2N + 0.00236P^2S - 0.00037TP^2 \\
& + 0.00169P^2N + 0.0000983PS^2 + 0.0000796TS^2 - 0.00000394S^2N - 0.00000222TN^2 \\
& + 0.0000543PN^2 + 0.00000042SN^2 + 0.000176T^3 - 0.0161P^3 + 0.0000217S^3 \\
& - 0.00000001N^3
\end{aligned}
$$

$r^2 = 0.880$

$$
\begin{aligned}
\mathrm{Ln(GT)} = {} & 30.1845 - 0.7674T - 10.0689P + 0.3615S + 0.0469N + 0.1019TP - 0.00466TS \\
& - 0.000565TN - 0.0758PS - 0.0142PN - 0.000264SN + 0.0114T^2 + 1.2803P^2 \\
& + 0.000393S^2 + 0.000198N^2 - 0.000193TPS + 0.0000308TPN - 0.00000309TSN \\
& + 0.0000482PSN - 0.000972T^2P + 0.0000597T^2S + 0.00000427T^2N + 0.00358P^2S \\
& - 0.00388TP^2 + 0.00118P^2N + 0.000523PS^2 + 0.0000471TS^2 + 0.00000318S^2N \\
& + 0.00000014TN^2 - 0.0000246PN^2 - 0.00000072SN^2 - 0.000031T^3 - 0.0546P^3 \\
& - 0.000061S^3 + 0.00000009N^3
\end{aligned}
$$

$r^2 = 0.913$

N, sodium nitrite (0–200 μg/ml); T, temperature (10–42°C); P, initial pH (4.5–8.5); S, sodium chloride (5–50 g/l).

Gompertz parameters

$$
\begin{aligned}
\mathrm{Ln}(B) = {} & -30.02213 + 0.6717T + 8.9831P - 0.0783S - 0.1093N - 0.0954TP - 0.000255TS \\
& - 0.0000796TN + 0.0436PS + 0.0248PN + 0.000258SN - 0.00649T^2 - 1.1242P^2 \\
& - 0.00366S^2 + 0.000198N^2 + 0.000726TPS - 0.00000637TPN - 0.00000053TSN \\
& - 0.0000644PSN + 0.000341T^2P - 0.000126T^2S + 0.00000242T^2N - 0.00409P^2S \\
& + 0.00553TP^2 - 0.0013P^2N - 0.000108PS^2 + 0.0000525TS^2 + 0.00000257S^2N \\
& + 0.00000021TN^2 - 0.000022PN^2 + 0.00000044SN^2 + 0.0000194T^3 + 0.0457P^3 \\
& + 0.0000311S^3 - 0.00000022N^3
\end{aligned}
$$

$r^2 = 0.918$

$$
\begin{aligned}
\mathrm{Ln}(M) = {} & 30.2349 - 0.5022T - 9.264P + 0.165S + 0.2322N + 0.0401TP - 0.0006988TS \\
& + 0.000671TN - 0.0367PS - 0.0549PN - 0.000477SN + 0.00708T^2 + 1.2115P^2 \\
& - 0.000234S^2 - 0.000438N^2 - 0.00027TPS - 0.0000703TPN + 0.00000272TSN \\
& + 0.00008PSN - 0.000543T^2P + 0.0000446T^2S - 0.00000438T^2N + 0.00247P^2S \\
& - 0.00126TP^2 + 0.00303P^2N + 0.000217PS^2 - 0.00000282TS^2 - 0.00000377S^2N \\
& - 0.00000026TN^2 + 0.0000556PN^2 + 0.00000003SN^2 + 0.00000799T^3 - 0.0518P^3 \\
& - 0.00000995S^3 + 0.00000031N^3
\end{aligned}
$$

$r^2 = 0.918$

Kinetics parameters

$$
\begin{aligned}
\mathrm{Ln(LPD)} = {} & 27.8469 - 0.3913T - 8.5394P + 0.141S + 0.3159N + 0.0184TP - 0.00133TS + 0.00104TN \\
& - 0.0119PS - 0.0753PN - 0.000515SN + 0.00548T^2 + 1.07P^2 - 0.00256S^2 \\
& - 0.000611N^2 - 0.00000083TPS - 0.00011TPN + 0.00000362TSN + 0.000082PSN \\
& - 0.000374T^2P - 0.0000344T^2S - 0.00000565T^2N + 0.000955P^2S - 0.000568TP^2 \\
& + 0.0041P^2N + 0.0000507PS^2 + 0.000062TS^2 - 0.00000478S^2N - 0.00000064TN^2 \\
& + 0.0000868PN^2 + 0.00000024SN^2 + 0.0000301T^3 - 0.0428P^3 + 0.0000101S^3 \\
& + 0.00000026N^3
\end{aligned}
$$

$r^2 = 0.869$

$$
\begin{aligned}
\mathrm{Ln(GT)} = {} & 42.8891 - 0.8217T - 15.0131P + 0.0749S + 0.106N + 0.1253TP - 0.000521TS \\
& + 0.000255TN - 0.0382PS - 0.025PN - 0.000247SN + 0.00804T^2 + 1.9481P^2 \\
& + 0.00387S^2 - 0.000168N^2 - 0.000812TPS - 0.00000221TPN + 0.000001TSN \\
& + 0.0000637PSN - 0.0003552T^2P + 0.00013T^2S - 0.00000485T^2N + 0.00361P^2S \\
& - 0.0067TP^2 + 0.00136P^2N + 0.00014PS^2 - 0.0000339TS^2 - 0.00000303S^2N \\
& - 0.0000003TN^2 + 0.000019PN^2 - 0.00000039SN^2 - 0.0000173T^3 - 0.0839P^3 \\
& - 0.0000402S^3 + 0.00000019N^3
\end{aligned}
$$

$r^2 = 0.916$

N, sodium nitrite (0–200 μg/ml); T, temperature (10–42°C); P, initial pH (4.5–8.5); S, sodium chloride (5–50 g/l).

For example, Fig. 3 depicts the effect of sodium chloride concentration for 28°C-pH 6.5 cultures containing 0 and 150 μg/ml $NaNO_2$.

Four sets of response surface models were generated for both the aerobic (Table 3) and anaerobic (Table 4) data sets. The models included: (1) quadratic models of Ln transformations of Gompertz B and M parameters (not shown); (2) quadratic models of Ln transformations of LPD and GT (not shown); (3) cubic models of Ln transformations of Gompertz B and M parameters; and (4) cubic models of Ln transformations of LPD and GT. The models of the Gompertz B and M parameters will be referred to as Gompertz parameters models, while those for LPD and GT will be referred to as kinetics parameters models. All four model types provided reasonable predictions for most combinations of the four cultural variables (temperature, pH, NaCl, and $NaNO_2$). As might be expected, the r^2 values for the cubic models were greater. The r^2 values for Gompertz parameters models tended to indicate a better fit than those for kinetics parameters models. However, comparison of predicted versus observed values for the aerobic (Fig. 4) and anaerobic (Fig. 5) data sets generally indicated that overall the most useful models were the cubic models of GT and LPD.

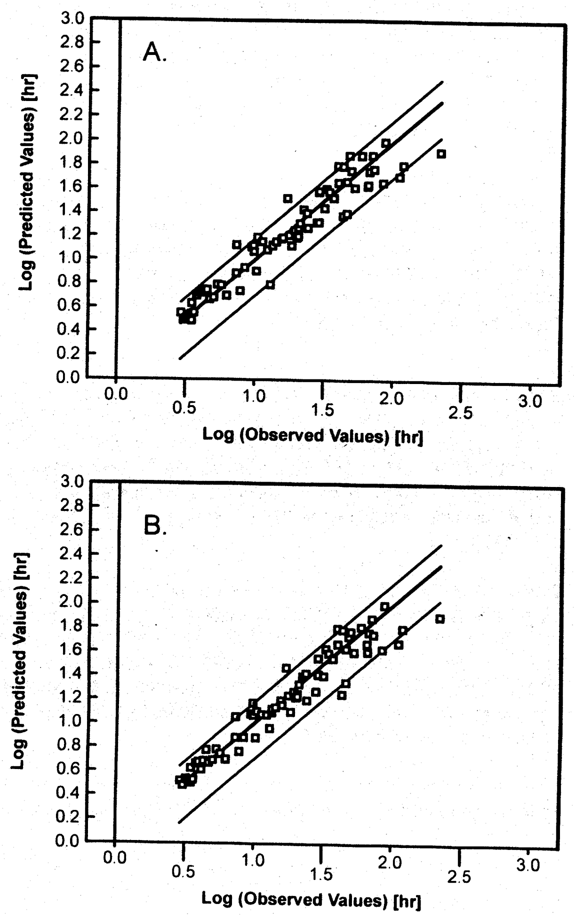

Fig. 4. Observed times for aerobic cultures of *Escherichia coli* O157:H7 to achieve 1000-fold increase in population density compared to those predicted by Ln transformations of (A) Gompertz parameters/cubic models and (B) kinetics parameters/cubic models.

One of the problems with generating response models based on the Gompertz *B* and *M* parameters is that when predicted growth kinetic values (i.e., LPD, EGR, and GT) are subsequently calculated, the prediction of negative LPD values is not unusual. This was noted using both the aerobic and anaerobic cubic models of Ln transformations of *B* and *M*. In the case of the aerobic data base, these occurred in conjunction with 5°C predictions. This actually represents an extrapolation of the models beyond the range that should be considered. While data were available for this temperature, the Ln transformation results in the 'no-growth' data being excluded from consideration during model generation. In this instance,

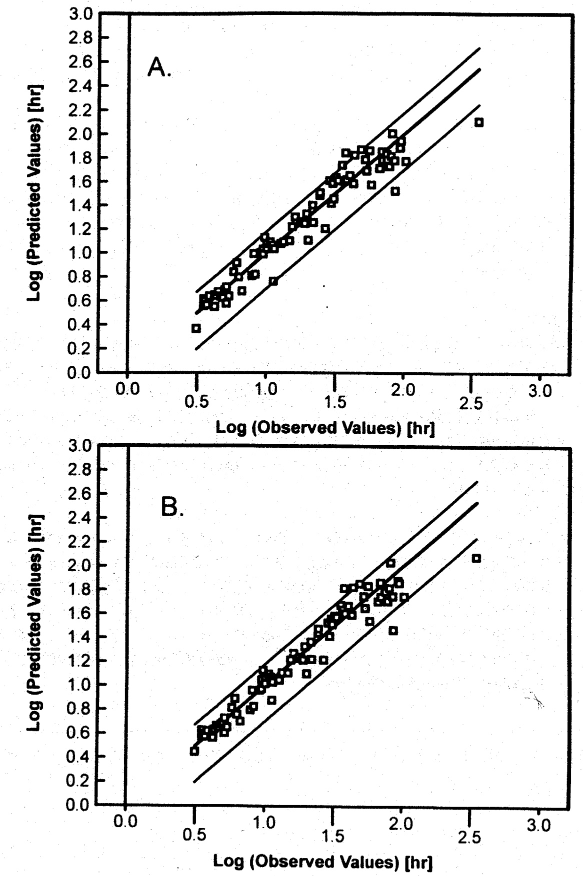

Fig. 5. Observed times for anaerobic cultures of *Escherichia coli* O157:H7 to achieve 1000-fold increase in population density compared to those predicted by Ln transformations of (A) Gompertz parameters/cubic models and (B) kinetics parameters/cubic models.

the anomalous values could be avoided by appropriately limiting the effective range of the model to temperatures ≥ 10°C. In the case of the anaerobic model, a negative LPD value was predicted for a single variable combination of 12°C-pH 7.5-50 g/l NaCl-0 μg/ml $NaNO_2$. This again represents a no-growth condition. In this instance the anomalous predictions would likely be corrected through the acquisition of additional data for variable combinations in this region of the data set.

Models based on both Gompertz parameters and kinetics parameters were generated, in part, to evaluate the suggestion of Garthright (1991) that the latter approach would be more effective. As indicated above, modeling the Gompertz parameters can result in difficulties with negative LPD values that may require

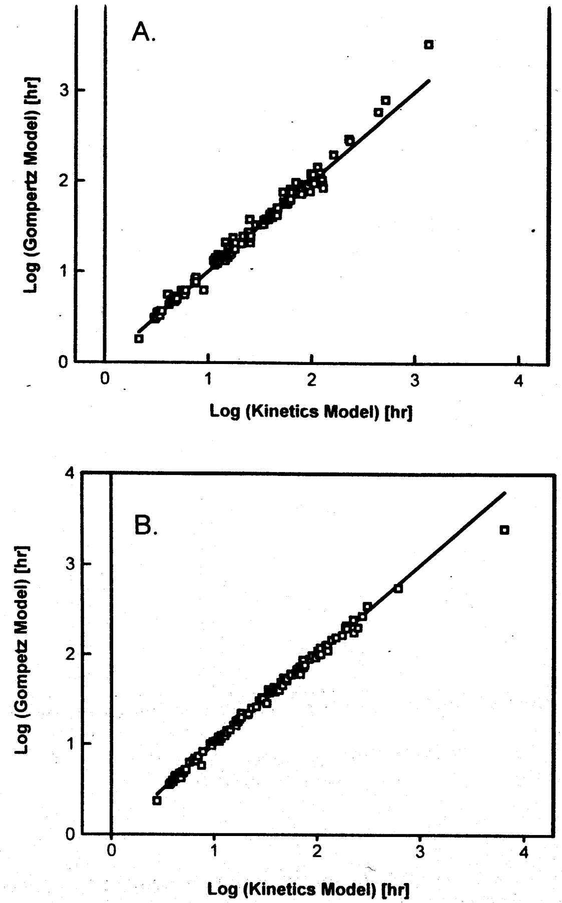

Fig. 6. Comparison of values predicted for the aerobic (A) and anaerobic (B) growth of *Escherichia coli* O157:H7 by cubic models based Ln transformations of the Gompertz parameters and the kinetics parameters. The variable combinations that did not support growth of the experimental cultures were excluded from consideration.

F-values for independent variables and their cross products for the cubic models based on the Ln transformations of the Gompertz *B* and *M* parameters and the Ln transformations of the lag phase duration (LPD) and generation time (GT) values

	Aerobic		Anaerobic	
	Ln(*B*)	Ln(*M*)	Ln(*B*)	Ln(*M*)
T	15.4[a]	7.3[b]	11.9[a]	9.7[b]
P	6.8[b]	3.7	7.6[b]	11.9[a]
S	14.7[a]	27.7[a]	0.7	4.6[c]
N	0.7	23.1[a]	9.9[a]	65.5[a]
TP	8.3[b]	5.4[c]	5.9[c]	1.5
TS	5.1[c]	0.5	0.0	0.2
TN	0.2	0.2	0.0	1.3
PS	8.9[b]	27.0[a]	4.3[c]	4.5[c]
PN	1.2	24.6[a]	8.2[b]	59.2[a]
SN	2.5	9.5[b]	0.9	4.4[c]
T^2	5.6[c]	0.0	2.7	4.7[c]
P^2	3.9	1.7	5.5[c]	9.4[b]
S^2	0.3	2.8	4.9[c]	0.0
N^2	2.1	0.5	2.7	19.0[a]
TPS	0.0	7.1[b]	10.9[b]	2.2
TPN	0.1	0.2	0.0	1.0
TSN	0.8	2.6	0.0	1.2
PSN	3.5	12.4[a]	4.6[c]	10.5[b]
T^2P	5.3[c]	1.3	1.0	3.7
T^2S	2.5	1.0	19.4[a]	3.5
T^2N	0.5	0.0	0.2	1.0
TP^2	5.1[c]	5.1[c]	5.3[c]	0.4
P^2S	1.5	19.4[a]	8.1[b]	4.3[c]
P^2N	2.7	17.7[a]	4.8[c]	38.6[a]
TS^2	2.0	8.7[a]	3.3	0.0
PS^2	9.4[b]	11.5[a]	0.5	3.0
S^2N	4.0[c]	0.1	1.0	2.9
TN^2	0.4	1.4	0.0	0.1
PN^2	1.8	1.6	1.6	15.3[a]
SN^2	3.5	0.3	1.1	0.0
T^3	0.1	5.4.[c]	0.2	0.1
P^3	1.9	0.4	3.6	6.8[b]
S^3	5.3[c]	2.3	3.1	0.5
N^3	0.3	0.0	1.1	3.2

additional data acquisition. However, after eliminating the single variable combination with a negative LPD value, comparisons of the predicted t_{1000} values for the two modeling approaches for the aerobic and anaerobic data sets indicated a high degree of correlation (Fig. 6). It appears that either approach can be effective if the models are derived from a significantly large data set and subsequently validated to ensure that there are no anomalous LPD predictions associated with specific variable combinations. The direct modeling of kinetics terms does appear to have advantages in relation to the amount of experimental data needed to generate an effective model. This potential advantage is being evaluated further using the data sets we have available for other foodborne pathogens (Buchanan and Phillips, 1990; Palumbo et al., 1991, 1992; Zaika et al., 1992; Buchanan et al., 1993b).

The *F*-values associated with the four independent variables and their cross products are summarized for both Gompertz parameters and kinetics parameters based response surface models (Table 5). In general, within the Gompertz parameters and kinetics parameters models the significant factors were similar for aerobic and anaerobic conditions. However, there was a great deal of difference in the significant variables between the Gompertz parameters and kinetics parameters

Table 5 (continued)

	Aerobic		Anaerobic	
	Ln(B)	Ln(M)	Ln(B)	Ln(M)
T	0.4	20.2 [a]	2.1	16.1 [a]
P	1.6	14.8 [a]	3.6	19.2 [a]
S	1.5	26.0 [a]	1.2	0.6
N	12.4 [a]	2.4	43.4 [a]	8.4 [b]
TP	1.0	8.4 [b]	0.1	9.2 [b]
TS	0.2	6.4 [c]	0.2	0.1
TN	1.1	1.0	1.1	0.1
PS	0.7	24.4 [a]	0.2	3.0
PN	10.8 [b]	3.7	40.0 [a]	7.6 [b]
SN	8.6 [b]	1.5	1.8	0.7
T^2	3.2	11.1 [b]	1.0	3.8
P^2	0.9	10.9 [b]	2.6	15.0 [a]
S^2	2.6	0.1	1.3	5.0 [c]
N^2	2.7	2.8	13.3 [a]	1.7
TPS	4.3 [c]	1.0	0.0	12.3 [a]
TPN	0.9	0.2	0.8	0.0
TSN	3.6	1.3	0.8	0.1
PSN	10.7 [b]	3.6	3.9 [c]	4.1 [c]
T^2P	0.0	9.6 [b]	0.6	2.4
T^2S	0.1	6.1 [c]	0.8	18.5 [a]
T^2N	0.0	0.9	0.6	0.8
TP^2	1.2	3.1	0.0	7.0 [b]
P^2S	1.8	9.7 [a]	0.2	5.7 [c]
P^2N	4.7 [c]	5.3 [c]	25.4 [a]	4.8 [c]
TS^2	4.3 [c]	3.5	2.4	1.2
PS^2	0.2	13.8 [a]	0.1	0.8
S^2N	1.3	2.0	1.7	1.2
TN^2	2.0	0.0	0.2	0.1
PN^2	4.7 [c]	2.2	13.4 [a]	1.1
SN^2	0.5	3.0	0.2	0.8
T^3	9.6 [b]	0.7	0.3	0.2
P^3	0.3	7.6 [b]	1.7	11.1 [b]
S^3	0.8	13.7 [a]	0.2	4.7 [c]
N^3	0.0	0.2	0.8	0.8

F-values based on type II sum of squares.
[a] $P < 0.001$.
[b] $0.01 \geq P \geq 0.001$.
[c] $0.05 \geq P > 0.01$.

models. Using the magnitude of the F-values as an estimate of the relative impact of the variables, both the aerobic and anaerobic models for Ln(B) had significant terms for temperature and at least two other primary variables, as well as several interaction terms. The models for Ln(M) were strongly dependent on a number of factors including several strong two- and three-way interaction associated with sodium nitrite. The aerobic and anaerobic models for Ln(LPD) were not dependent on the primary variables except sodium nitrite. The Ln(LPD) models had several significant interaction terms, including strong interaction terms of pH X nitrite. Alternatively, the Ln(GT) models had significant linear terms for temperature, pH, and sodium chloride, as well as significant square or cubic terms for different primary variables. There were also several moderately significant interaction terms. Previous model development for the effects of temperature, pH, and sodium chloride on the growth *E. coli* O157:H7 had concluded that quadratic models were sufficient to describe the impacts of the three variables (Buchanan et al., 1993a). However, substantially improved fits were achieved in the current study by employing a cubic model when sodium nitrite was included as a variable. The above analysis of F-values suggests that this is due to the bacteriostatic activity of sodium nitrite being dependent on its interaction with the other variables.

Table 6
Comparison of predicted and observed times for *Escherichia coli* O157:H7 to achieve a 1000-fold increase in population density as calculated from data reported by Glass et al. (1992) and Buchanan et al. (1993)

	Temperature (°C)	pH	NaCl (%)	Time (h) to 10^3 increase	
				Observed	Predicted [a]
Canned tuna [c]	42	5.9	1.5 [b]	4.3	3.6
Canned dogfood [c]	12	6.6	1.5 [b]	54.3	50.9
Chicken broth [c]	28	6.0	1.5 [b]	5.1	4.8
UHT milk [c]	19	6.5	0.5 [b]	16.6	12.3
Tryptic soy broth [d]	37	7.3	0.5	< 3.9	3.1
		7.3	1.5	< 3.9	2.8
		7.3	2.5	4.1	3.1
		7.3	4.5	11.5	8.6
		7.0	0.5	< 3.9	3.1
		6.5	0.5	< 3.9	3.0
		6.0	0.5	< 3.9	3.1
		5.5	0.5	5.4	3.8
		4.5	0.5	7.4	4.8

[a] Predicted using the cubic kinetics-parameters models for aerobic growth (see Table 3).
[b] Value assumed.
[c] Calculated using the values reported by Buchanan et al. (1993).
[d] Calculated using the values reported by Glass et al. (1992).

As mentioned above, the cubic kinetics parameters models were deemed to provide the best overall fit with the observed data. These models were internally validated for the cultural system employed by performing the experimentation in an iterative manner. Validation of the models against food data is hampered by a lack of quantitative data for the growth of O157:H7 strains in foods, particularly those containing sodium nitrite. However, comparisons of predicted t_{1000} times against those calculated from the growth kinetics data reported by (Glass et al., 1992; Buchanan et al., 1993a) indicate that the models are highly effective (Table 6) for making first estimates of the growth characteristics of *E. coli* O157:H7. Regretfully, no data appear to have been reported on the effects of sodium nitrite on the growth of *E. coli* O157:H7 in foods or other model systems. Additional studies are needed to verify the effectiveness of the model in predicting the growth of the pathogen in a number of foods. One of the factors that needs to be considered in that work is whether the initial or residual sodium nitrite concentration is the more appropriate value for measuring the impact of the curing agent on the growth of this or other susceptible foodborne pathogens. While additional validation is required, the models developed in the current study do appear to provide an effective means for rapidly acquiring initial estimates of the effects of temperature, pH, sodium chloride content, sodium nitrite concentration, and oxygen availability on the growth of *E. coli* O157:H7.

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